BOREAS An international journal of Quaternary research

Homotherium serum and *Cervalces* from the Great Lakes Region, USA: geochronology, morphology and ancient DNA

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Widga, C., Fulton, T. L., Martin, L. D. & Shapiro, B. 2012 (October): *Homotherium serum* and *Cervalces* from the Great Lakes Region, USA: geochronology, morphology and ancient DNA. *Boreas*, Vol. 41, pp. 546–556. 10.1111/j.1502-3885.2012.00267.x. ISSN 0300-9483.

A scimitar-toothed cat (*Homotherium serum*) and stag moose (*Cervalces* sp.) are described from Tyson Spring Cave, Fillmore County, Minnesota. These specimens represent the first records of both species in the state, and the first record for *H. serum* in the Great Lakes region. Although the *Cervalces* specimen remains undated, it shares features with pre-Wisconsin specimens from the eastern Great Plains. The *H. serum* individual dates to *c.* 26.9 ka, when the Wisconsin ice margin was less than 60 km away. Genetic analyses support the identity of the *Homotherium* specimen as conspecific with *Homotherium serum* found in older Beringian deposits, as well as both the early divergence of tribes Homotherini and Smilodontini within Machairodontinae and the early divergence of this Machairodontinae from the lineage that produced extant cats.

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The Driftless Area of the upper Midwestern United States covers parts of Minnesota, Wisconsin, Iowa and Illinois (Fig.1). Despite its name, only the eastern portion of the region is truly free of glacial deposits. The bedrock-dominated landscape of southern Minnesota and northeastern Iowa exhibits remnants of pre-Illinoian till and glaciofluvial deposits (Leverett 1932; Hallberg *et al*. 1984). The landscape is highly dissected, and surface outcrops of Palaeozoic bedrock, post-Illinoian loess, and pre-Wisconsinan drift are common (Hallberg *et al*. 1984; Leigh & Knox 1994). The land surface was not glaciated during the Wisconsinan glaciation and exhibits a variety of micro-habitats, including some that are unique to modern North America (Nekola 1999).

Karst features are common on this landscape. Caves, blind valleys and sinkholes are distributed throughout the region (Hogberg & Bayer 1967; Bounk & Bettis 1984). Most solution caverns in the area are formed in upper Ordovician carbonates of the Galena group (Alexander & Lively 1995; Runkel *et al*. 2003). Although there is the potential for pre-Quaternary palaeokarst, karst formation generally occurred during the Pleistocene (Bounk 1983; Bounk & Bettis 1984). Speleothem activation is episodic and generally related to regional climate conditions (Schwarcz *et al*. 1976; Denniston *et al*. 1999; Baker *et al*. 2002). U-series dating of speleothem activity in southeastern Minnesota caves suggests warmer and wetter conditions from 163 to 100 ka, from 60 to 35 ka, and from 15 ka to present, corresponding to interglacial or interstadial conditions (Lively 1983; Dorale *et al*. 1998). Few samples indicate the presence of open cave systems prior to 250 ka.

The 'Lead district' of Illinois provided paleontological material described by Leidy (1869) and J. LeConte (1848), including *Platygonus compressus* (type), *Megalonyx jeffersoni*, *Bison* sp., and smaller fauna from a lead-bearing crevice. Despite additional geological fieldwork in the early 20th century (Leverett 1932), there was no additional substantive work on the Quaternary vertebrates of the region until Stauffer's (1945) review of Ice Age mammals in southeastern Minnesota. He documented a number of taxa from the region's sand and gravel quarries, including *Mammuthus* (*Elephas*) *jeffersonii*, *M.* (*Elephas*) *primigenius*, *Mammut americanum* and *Bootherium bombifrons*.

Quaternary small-mammal assemblages are also known from the region. The Moscow Fissure local fauna is a small-mammal assemblage from southwestern Wisconsin with nine extralimital taxa, including *Thomomys talpoides*, *Dicrostonyx torquatus*, *Microtus xanthognathus*, *Zapus princeps*, *Synaptomys borealis* and *Phenacomys intermedius* (Foley 1984). An associated ^{14}C date indicates that this assemblage accumulated during the Last Glacial Maximum (LGM). An additional LGM small-mammal assemblage from Elkader, IA (Woodman *et al*. 1996) also contains arctic/sub-arctic taxa (i.e. *Spermophilus parryii*, *Dicrostonyx torquatus* and *Microtus* cf. *miurus*). Isolated Quaternary vertebrates from the Driftless Area include a peccary canine from Castle Rock Cave, Grant County, WI (Palmer 1974) and caribou remains from Bogus Cave, Jones County, IA (Josephs 2005).

DOI 10.1111/j.1502-3885.2012.00267.x

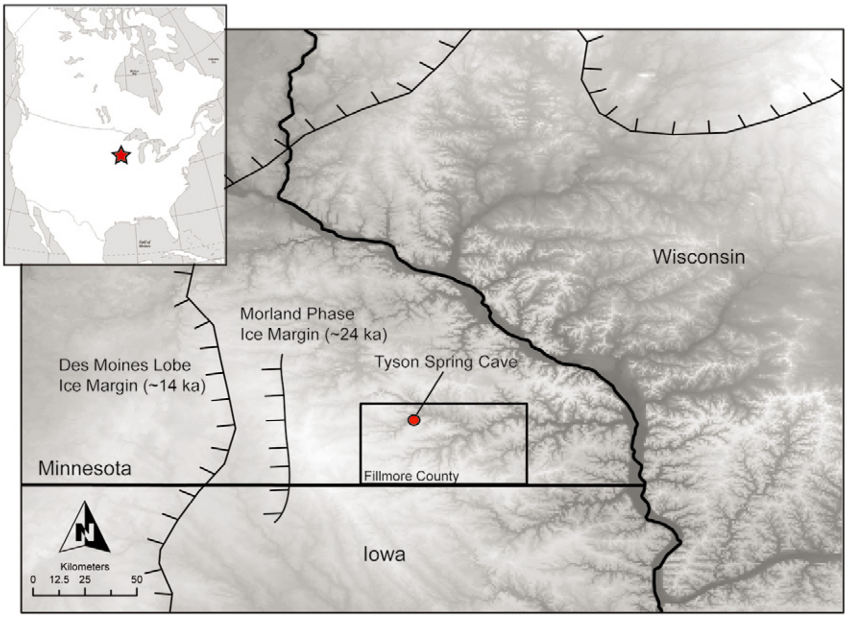

Fig. 1. Map showing the location of Tyson Spring Cave and the approximate glacial coverage in the upper Midwest at 22 ka (modified from Meyer & Knaeble 1998). This figure is available in colour at http://www.boreas.dk.

This study describes the first occurrence of *Homotherium serum* in the Great Lakes region, and the first *Cervalces* specimen from Minnesota (USA). Additional palaeontological, geochronological and ancient DNA studies contribute to the clarification of phylogenetic and palaeoecological relationships in North American Machairodontinae.

Materials and methods

The karst area of southeastern Minnesota and northeastern Iowa is largely confined to Ordovician-aged carbonate rocks with numerous known cave systems (Hogberg & Bayer 1967). Most palaeoecological work in this area has focused on cave speleothem records of late Pleistocene and Holocene climate change (Lively *et al*. 1981; Denniston *et al*. 1999; Baker *et al*. 2002). Although small-mammal Quaternary faunas are locally present within these karst formations (e.g. Foley 1984), large mammals are extremely rare (Palmer 1974; Josephs 2005).

Tyson Spring Cave (TSC; Fillmore County, MN; Fig. 2) is a solution cavern in the Driftless Area of southeastern Minnesota formed in Ordovician limestone of the Galena group (Mossler 1995). This cave is currently being explored and mapped by the Minnesota Karst Preserve, a non-profit organization dedicated to the conservation and exploration of Minnesota caves. TSC was discovered in the late 19th century, but exploration was discouraged because of an underwater entrance until 2006, when the Minnesota Karst Preserve created artificial access. Compared with other caves in the region, TSC is a relatively large, low-gradient, dendritic conduit (Runkel *et al*. 2003: p.103) with an estimated length of 5–8 km. In 2008, John Ackerman and Clay Kraus recovered the cranium of a large cervid while excavating a side passage ~0.5 km downstream of the cave's artificial entrance. This specimen was brought to the attention of researchers at the Minnesota Historical Society and the Illinois State Museum, who identified it as *Cervalces* sp. Additional vertebrate remains were recovered on two subsequent trips to the cave, including the *H. serum* material described herein. All specimens from TSC were prepared and stabilized at the Illinois State Museum; however, the repository of record is the Science Museum of Minnesota, St Paul, MN (SMM).

TSC is a wet cave. The hydrology of the cave is directly linked to surface precipitation and it can flood quickly. Flowstone formations are common

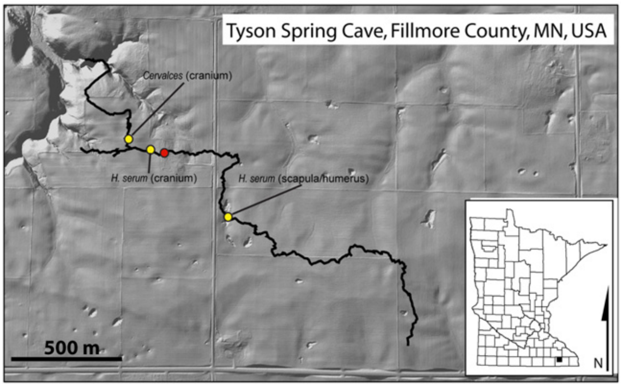

Fig. 2. Map of Tyson Spring Cave showing the location of *Cervalces* and *H. serum* finds. Map courtesy of M. Covington, Karst Research Institute. This figure is available in colour at http://www.boreas.dk.

throughout the cave, although in the vicinity of both vertebrate finds the channel flows on a consolidated gravel base or thalweg (Bosch & White 2007). Fine-grained channel deposits (fine gravel or coarse sand) are also present in the cave, usually on the downstream side of large rock falls.

Comparisons were made with specimens of extinct and extant taxa in the Illinois State Museum (ISM), University of Nebraska State Museum (UNSM), Field Museum of Chicago (FM) and University of Kansas Natural History Museum (KUNHM). Morphological comparisons were made with crania of *Xenosmilus hodsonae* (cast) from Alachua County, FL; *Smilodon fatalis* (cast) from Texas; *Homotherium ischyrus* (cast) from Texas; *Homotherium serum* (cast) from Friesenhahn Cave, TX; *Panthera spelaea* (cast) from Alaska; and *Panthera atrox* from Natural Trap Cave, WY. Extant specimens used for comparison include *Panthera tigris* (cast) and *Panthera leo*. Additional post-cranial elements from extant and extinct taxa in the collections of the ISM and FM were measured for comparison with the TSC *H. serum*. Specimens of extinct and modern Cervidae from the ISM, FM and UNSM were used for comparison with the TSC *Cervalces* cranium. Standardized measurements follow von den Driesch (1976) unless otherwise specified. Measurements were collected using standard dial calipers for distances. Photographs were taken with a Nikon D60 digital SLR, and the image analysis software MorphoJ (Klingenberg 2011) was used to capture angular data.

DNA isolation, amplification and sequencing

DNA extraction and all pre-PCR work of the *Homotherium* specimen was performed in a dedicated ancient DNA (aDNA) facility at The Pennsylvania State University that is housed in a separate building from any laboratories that perform genetic analysis. Ancient DNA protocols were strictly adhered to at all stages, including, but not limited to, the use of full body suits and face masks, sterile reagents and plasticware, sterilization of surfaces and equipment with bleach and ethanol, and one-way movement of reagents, supplies and workers, preventing any PCR-contaminated material from entering the facility (Cooper & Poinar 2000; Gilbert *et al.* 2005). Prior to processing the *Homotherium* specimen described here, no felid specimens had been processed in this facility.

DNA was extracted from the cranial bone following the protocol described in Rohland *et al.* (2010), with a final elution into 50 μL TE plus 1.5 μL Tween20. The specimen was given a lab extraction ID of TF76. An extraction negative control (no sample) was carried out simultaneously. A second *Homotherium* individual (Canadian Museum of Nature, CMN 46442) was also extracted (lab extraction ID: TF99) and sequenced following the processing of TF76. Owing to the level of DNA fragmentation in ancient samples, the target DNA sequence was amplified in a series of short, overlapping fragments using both previously published (Barnett *et al.* 2005) and newly designed primers (Table S1 in Supporting Information). For both samples, a 311-base-pair (bp) region of mitochondrial *cytochrome b* (cytb) was obtained in three overlapping amplifications, plus a short nested fragment (Homo_1F/Homo_4R; Barnett *et al.* 2005) to ensure that independent amplifications with different primer sets recovered the same sequence. We obtained a 142-bp region of ATP8 from TF76 with one amplification, and 279 bp of 12S from TF99 with two (Table S1). PCR conditions can be found in Table S1. Negative PCR reactions (containing no DNA extract) were included for each amplification reaction. PCR products were cleaned using Millipore Multiscreen PCRμ96 filter plates. Most fragments were amplified and cloned twice to ensure reliable sequence. Cloning was performed using a reduced-volume reaction of the TOPO-TA cloning kit (Life Sciences). Multiple clones were amplified in a 12.5-μL total reaction volume comprising 1× Econotaq buffer (including $MgCl_2$), 0.625 U Econotaq (Lucigen), 0.25 mM dNTPs, 1 μM each M13F(-20) and M13R, and 5 μL of the colony in water (each colony was picked into 50 μL of water). Positive clone amplifications were sequenced with BigDye 3.1 chemistry (Life Sciences) using M13F(-20). Following ethanol/EDTA precipitation, sequences were resolved on an *ABI3730xl* DNA Analyzer (Applied Biosystems) at the Penn State Genomics Core Facility (University Park). All sequences were visualized and aligned using Lasergene 8 (DNASTAR).

Molecular phylogenetic analysis

All available Feliformia cytb sequence was downloaded from GenBank and aligned using mafft 6 (Katoh & Toh 2008). Alignments were adjusted by eye and trimmed to a similar length to the *Homotherium* sequences using Se-Al 2.0a11 (Rambaut 2002). Sequences that did not cluster with other sequences from the same species in preliminary analyses were marked as putative nuclear insertions of mitochondrial DNA (numts) and, to be conservative, excluded from further analysis. The number of individuals per species was randomly re-sampled down to seven or fewer within Felidae or two for genera outside Felidae, and duplicate haplotypes were removed. Combined analyses of cytb, 12S and ATP8 were performed, including the 17 species for which sequence was available for these genes. GenBank accession numbers are listed in Table S2. In most cases, the same individual was used for all three genes. For both the cytb-only (multiple individuals per species) and all-genes-combined (one individual per species) data sets, maximum likelihood bootstrap (MLBP) analysis in RAxML 7.0.4 (Stamatakis 2006) and Bayesian

Fig. 3. *Cervalces* sp. cranium from Tyson Spring Cave. A) dorsal, B) ventral. This figure is available in colour at http://www.boreas.dk.

estimation in MRBAYES 3.2 (Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004) were performed. A full maximum likelihood search and 100 rapid bootstrap pseudo-replicates (-f a) were performed in RAxML using the GTRGAMMA model of evolution. The combined analysis was performed with each gene assigned to a separate partition. Two MRBAYES runs of 7 million generations each were performed for the cytb-only data set, while two runs of 5 million generations were performed for the combined data set. The *sump* command in MRBAYES was used to visualize that for each analysis the two runs had converged on the same distribution and that the potential scale reduction factor for all parameters was 1.0 following removal of a 10% burn-in. The best-fit model of evolution as selected by AIC in JMODELTEST 0.1 (Posada 2008) was applied for each gene separately (cytb: HKY+G; 12S: GTR+I+G; ATP8: GTR+I), and all evolutionary model parameters were unlinked between partitions.

Description of specimens

Cervalces sp.

A partial cranium (SMM P2012:1:1; Fig. 3; Table S3) was uncovered during excavation of a side passage between the 'wimp room' and the new, artificial entrance. The isolated specimen was found in slackwater facies (silt) at the entrance to the side passage. Subsequent examination of the sediment bank and exploratory screening through 1.6-mm mesh did not yield additional remains, and loss-on-ignition analyses of the sediment itself indicate minimal organic preservation. The entire surface of this specimen is partially covered by small gravels cemented to the bone by a carbonate layer. Efforts to direct-date the specimen failed to yield adequate collagen for ^{14}C dating.

Although not uncommon in neighbouring Midwestern states (Churcher & Pinsof 1988; Schubert *et al.* 2004; Long & Yahnke 2011), the *Cervalces* cranium from TSC is the first record of this taxon in Minnesota. This cranium consists of a relatively complete right frontal and the attached distal beam of the antler. The antler extends laterally from the skull at ~100° (relative to the skull midline) for 247 mm. Overall, the beam of the antler is relatively straight, elliptical in cross-section, and lacking a brow tine, unlike *Rangifer*, *Cervus* and *Odocoileus*. The distance between the pedicle and the end of the beam shows no palmate development, whereas in *Alces alces* palmation occurs a short distance above the pedicle. We refrain from a specific diagnosis because there are minor differences between this specimen and most *C. scotti* material from the Great Lakes and eastern United States (e.g. Riggs 1936; Churcher & Pinsof 1988; McDonald 1989). Although the TSC beam is damaged, it has a longer pre-palmate extension than is typically found in other Great Lake *C. scotti*. Furthermore, the frontal region of the antler pedicle of *C. scotti*, as expressed in other regional specimens, is dorsoventrally rounded to accommodate the pressure of large antlers. However, this area of the TSC specimen is more strongly integrated into the frontal structure. The frontal surface is smooth and lacks the distinctive bump where the antler attaches to the skull. Both the smooth frontal region and the long beam of the antlers are features shared with specimens from central Nebraska and western Iowa, including the type specimen of *C. roosevelti* (Hay) from Denison, Crawford County, IA (Hay 1913). Both specimens were recovered from contexts that are probably pre-Wisconsin (Hay 1913; Schultz & Hillerud 1977). Cook (1931) noted the presence of *C. roosevelti* (Hay) from a gravel pit near Giltner, Hamilton County, NE. He drew attention to the long antler beam of this specimen, longer than that of either *C. scotti* or *C. borealis*. Further examination of this specimen (UNSM 3/12/28-cast) verifies the long beam of the antlers and the absence of a frontal bump where they attach to the skull. Churcher & Pinsof (1988) assigned this specimen to *C. scotti* on the basis of its antler measurements. We do not feel confident in a specific assignment of the Giltner and TSC *Cervalces* material, nor of the synonomy of *C. scotti* and *C. roosevelti*. It is conceivable that the latter represent an earlier stage of *Cervalces* in the Mid-Continent. The age of the *C. roosevelti* type specimen is pre-Wisconsin (Aftonian), and the Giltner quarry has been suggested to be Illinoian (Schultz & Hillerud 1977). A pre-Wisconsin age remains possible for the TSC specimen given its context within the Driftless Area.

Homotherium serum

Three elements from an individual *Homotherium serum* were recovered from the middle reaches of TSC: a partial cranium (SMM P2012:2:1), left scapula, (SMM P2012:2:2), and right humerus, (SMM P2012:2:3). All bones were found *ex situ* on the floor of the cave, although only the cranium exhibits breakage or wear indicative of stream transport. The cranium was discovered ~15 m downstream from the entrance ladder, in a sandy deposit at the edge of the channel. This location was immediately downstream from a 1–2 m thick deposit of sandy gravel. The humerus and scapula were recovered on subsequent visits to the cave at the base of a high dome, ~0.5 km upstream from the cranium.

A sample of the petrosal was submitted to GNS Scientific, Rafter Radiocarbon Laboratory in New Zealand for collagen extraction and subsequent ^{14}C analyses of bone gelatin and stable isotope analyses. The resulting age of this specimen is 22 250±130 ^{14}C a BP (NZA 30409), with a 2-sigma calibrated age range of 27 590–26 200 cal. a BP (calibrated in OXCAL 4.1 (Bronk Ramsey 2009) using the Intcal09 dataset (Reimer *et al.* 2009)). The C:N ratio of extracted collagen is 3.4 and within the range of modern, un-fossilized bone, as is the %N-value (13.5%), which indicates that collagen was well preserved. A relatively high degree of confidence can be put in this ^{14}C date.

The chronology of North American *H. serum* is uncertain. It is absent in Beringia after 36 000 a BP (Fox-Dobbs *et al.* 2008) and may also have a mid-Wisconsinan terminal age in much of western North America (Dundas 1992; Jefferson & Tejada-Flores 1993). Isolated *H. serum* specimens are present in collections from Rancho la Brea (Los Angeles Co., CA), but cannot be associated with known-age horizons (Jefferson & Tejada-Flores 1993). Rancho la Brea deposits probably date between >45 000 and 11 000 a BP (Marcus & Berger 1984; Friscia *et al.* 2008). Efforts to date the Friesenhahn Cave assemblage (Bexar Co., TX) have also yielded mixed results. Although ^{14}C-dated collagen from the 1970s suggested an LGM age for the deposit (Graham 1976), subsequent dating efforts and improved collagen extraction methods have been unsuccessful (Graham 2007). Recently, Schubert (2010) reported a date of 10 814±55 ^{14}C a BP on *Arctodus simus* tooth dentin from the locality. The age of this specimen should be approached with caution owing to the relatively poor protein preservation.

Cranium. – The cranium was submerged, ventral side up, in sandy gravel. The posterior portion of the specimen is preserved, from the anterior frontal to the occipital crest, including a well-preserved braincase (Fig. 4; Table S4). It lacks all elements anterior to the orbits, including teeth. The occipital condyles are also broken, and the auditory bullae are damaged. Morphologically, the skull shows affinities to machairodont cats, especially large, late Pleistocene specimens of dirk- and scimitar-toothed cats.

The frontals in this specimen are wide relative to the size of the skull, similar to homothere frontal elements, which are wide and short. *Panthera atrox* (NTC), *Xenosmilus* and *Panthera tigris* exhibit long, narrow frontals. *Smilodon* and *Panthera spelaea* are intermediate in this respect. Relative to other measures of skull size, homothere sagittal crest length is reduced compared with other machairodonts and Pantherines. The sagittal crest in the TSC specimen is slightly convex, but significantly shorter than the sagittal crest in *Smilodon*, *Xenosmilus*, *P. atrox* or *P. spelaea* of similar size. The occipital region in this skull is relatively narrow, but exaggerated dorso-ventally. The angle between the sagittal crest and the occipital is moderate in this specimen, as in other machairodonts. This angle in Pantherines (*P. tigris*, *P. atrox*, *P. spelaea*) is more acute. In the basio-occipital region, the TSC specimen exhibits a bridge between the antero-medial edge of the auditory bullae and the glenoid peduncle. In this specimen and other homotheres, this bridge is relatively deep, and the external auditory meatus tilts downwards medio-laterally. In *Panthera*, this bridge is low and extends only a short distance to the external auditory meatus, which faces directly lateral.

Scapula. – Most of a left scapula was recovered from TSC (Fig. 5; Table S5). This specimen lacks the extreme proximal border, but much of the glenoid, blade and spine are intact. In comparison to *Panthera atrox* (NTC), the glenoid facet is flattened medio-laterally and the acromial process is elongated antero-posteriorly. There is also a broad sulcus present along the posterior border of the distal spine. This feature is absent in *Panthera*.

This morphology for *H. serum* is further supported when the analysis is extended to other large members of the Felidae (Fig. 6). The medio-laterally compressed glenoid is similar to other *H. serum* specimens; however, the TSC specimen and a single Friesenhahn Cave specimen are less compressed than glenoids of other homotheres. The angle between the scapular ridge and posterior border is also more acute in *H. serum* (~18°) than in any of the other measured taxa.

Humerus. – The right humerus from TSC is complete except for the proximal extremity (Fig. 7; Table S6). This element is slender and elongated. The anterior crest of the diaphysis has migrated proximally as a result of this elongation. The TSC humerus is more elongate than in *Smilodon*, *Xenosmilus* or *Panthera*, and exhibits a relatively small entepicondylar foramen, as in other homotheres. The depression above the anterior margin of the distal condyle is smoothly triangular, as opposed to the case in *Panthera* and *Smilodon*, where this area is roughened and lacks a distinctive shape. The anterior trochlea is more medio-laterally compressed

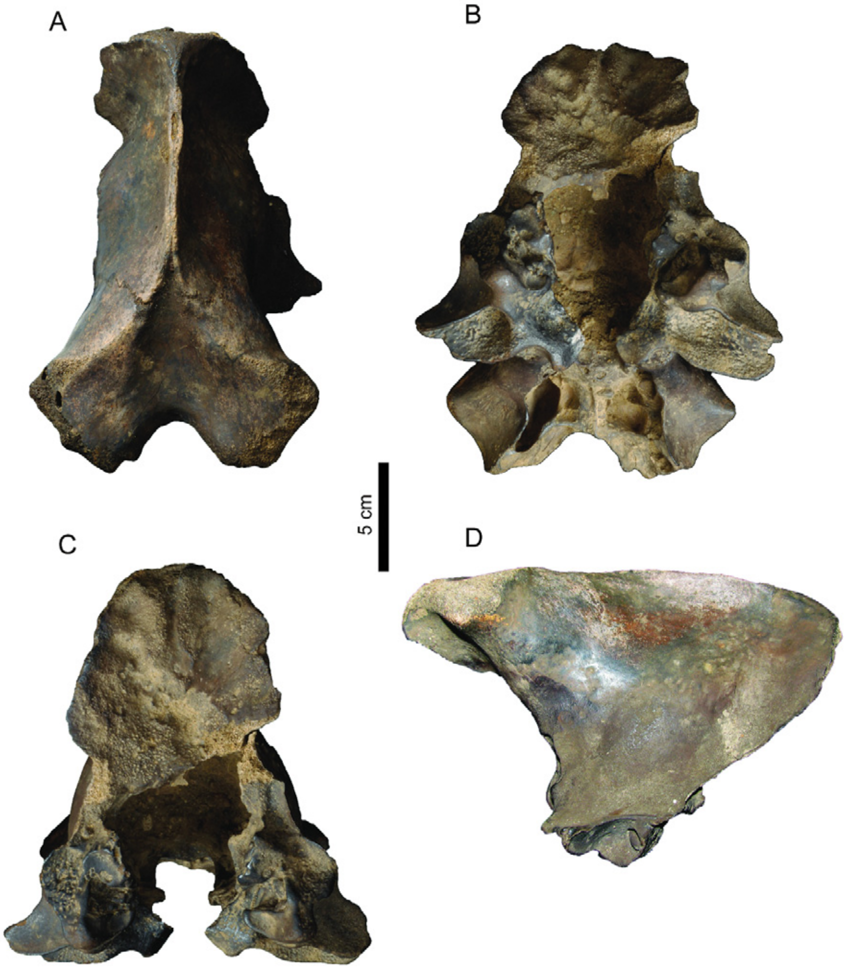

Fig. 4. *H. serum* cranium from Tyson Spring Cave. A) dorsal, B) ventral, C) posterior, D) left side. This figure is available in colour at http://www.boreas.dk.

than in *Panthera* or *Smilodon*, indicative of a more cursorial lifestyle. *Panthera* also exhibits a sulcus along the lateral edge of the distal diaphysis. This feature is absent or reduced in homotheres. Compared with other large-cat samples (i.e. *Smilodon, Panthera atrox*), *H. serum* humeri are long and slender (Fig. 8). Relative to its breadth, the TSC specimen is longer than any of the Friesenhahn Cave individuals.

Genetic results

A 311-bp region of cytb obtained from the *Homotherium* specimen (sequence extract TF76) differed by only 2 bp from the cytb sequence in another *Homotherium serum* specimen (CMN 46442; sequence extract TF99), confirming the species identification of this specimen. This region of cytb in CMN 46442 was sequenced previously from a different DNA extraction using different primers (Barnett *et al*. 2005). Our sequence result for CMN 46442 (TF99) was an identical match to this sequence (GenBank DQ097176), providing independent evidence for its authenticity.

Fig. 5. *H. serum* scapula from Tyson Spring Cave. A) lateral, B) medial, C) glenoid. This figure is available in colour at http://www.boreas.dk.

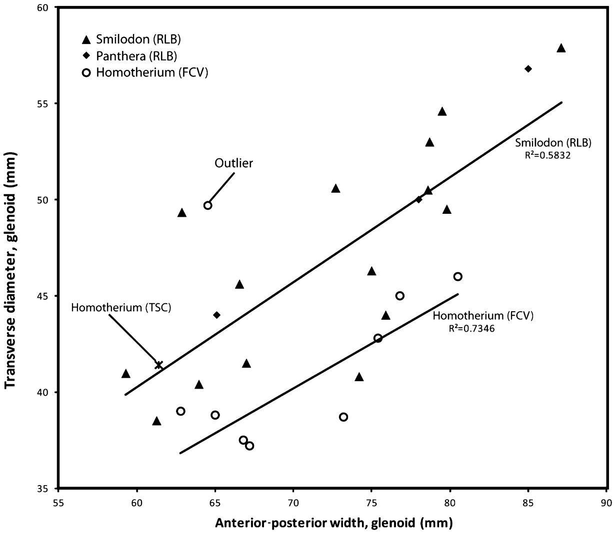

Fig. 6. Measurements on scapulae from *P. atrox*, *Smilodon* and *H. serum*. RLB = Rancho la Brea (Merriam & Stock 1932); FCV = Friesenhahn Cave (Rawn-Schatzinger 1992); TSC = Tyson Spring Cave.

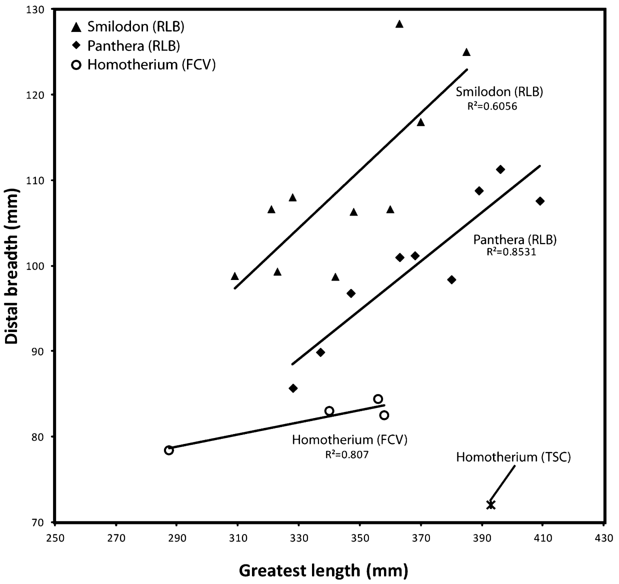

Fig. 8. Measurements on humeri from *P. atrox*, *Smilodon* and *H. serum*. RLB = Rancho la Brea (Merriam & Stock 1932); FCV = Friesenhahn Cave (Rawn-Schatzinger 1992); TSC = Tyson Spring Cave.

To place *Homotherium* within a molecular phylogenetic framework, 140 bp of mitochondrial ATP8 was obtained from the newly described specimen TF76, and a 279-bp region of 12S was obtained from TF99 (CMN 46442). Newly obtained sequences have been entered into GenBank under the accessions JQ937107–9. A region of 16S was also targeted, and, although cloning and sequencing of three overlapping amplifications all produced 16S rRNA-like sequences, mismatches occurred between fragments, indicating that one or more of the amplifications was a numt, an extremely common occurrence in felids (Davis *et al.* 2010). Therefore, 16S sequences were not included in any further analysis.

The two *Homotherium* cytb sequences were strongly supported as sister taxa (Fig. 9A), and *Homotherium* was recovered as the sister species to *Smilodon* in both cytb-only and combined gene analyses (Fig. 9). The *Homotherium–Smilodon* lineage is supported as belonging to the Felidae lineage and diverges prior to the diversification of extant felids (Fig. 9A).

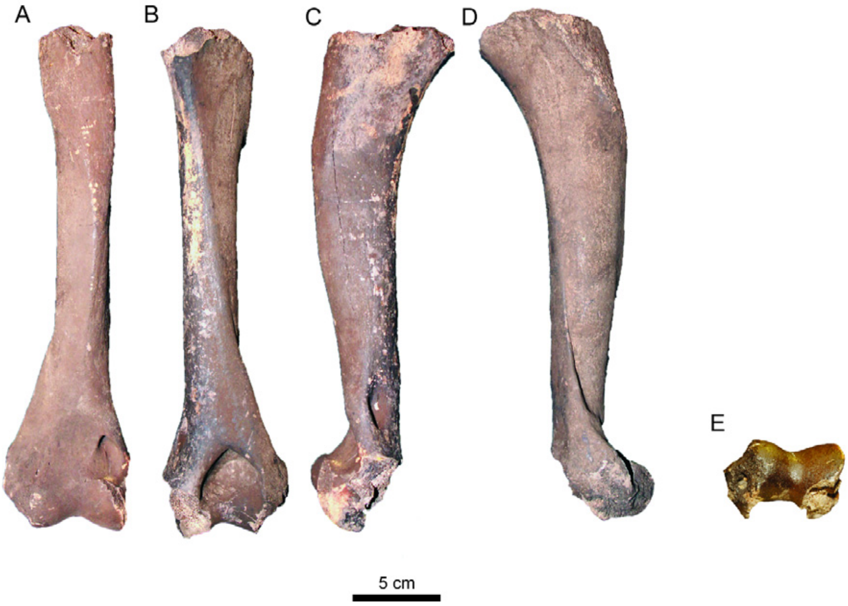

Fig. 7. *H. serum* humerus from Tyson Spring Cave. A) anterior, B) posterior, C) medial, D) lateral, E) distal. This figure is available in colour at http://www.boreas.dk.

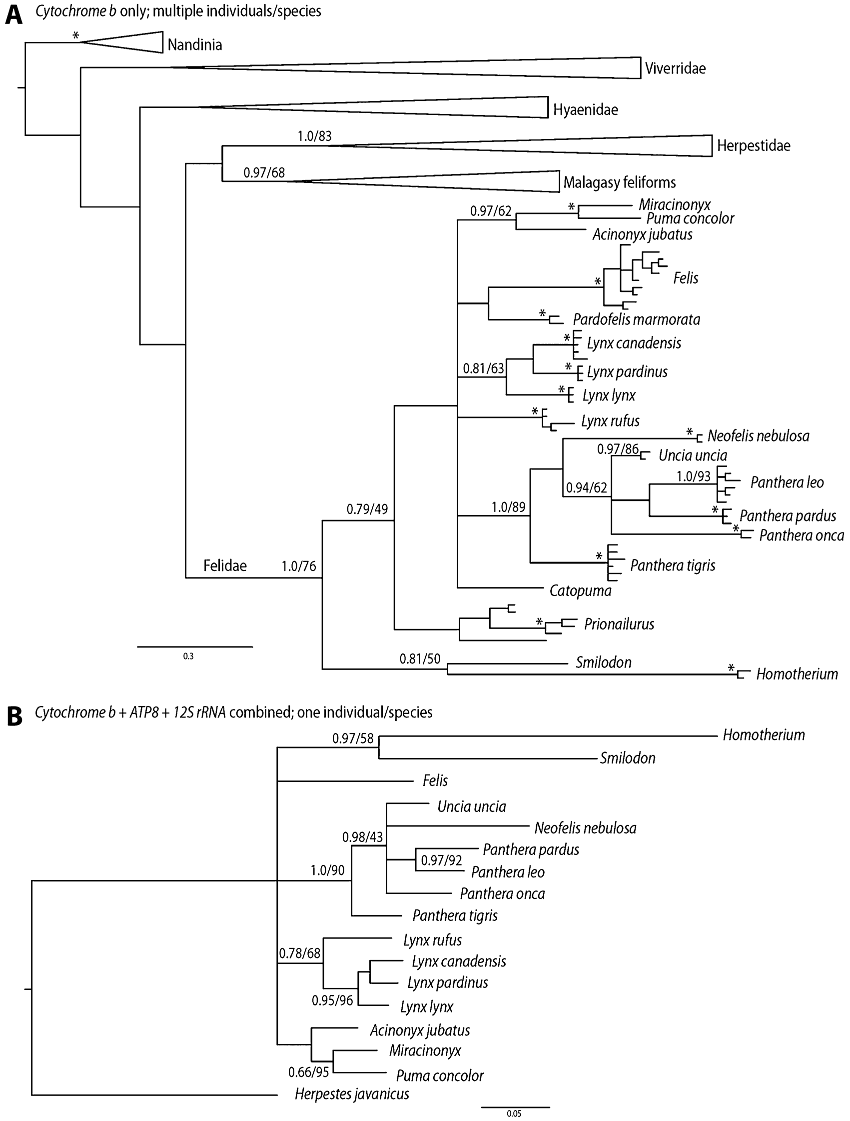

Fig. 9. Molecular phylogenetic placement of *Homotherium* within Feliformia. Bayesian phylogeny of (A) extant feliforms plus *Homotherium*, *Smilodon* and *Miracinonyx* based on *cytochrome b* data, including multiple individuals per species; clades outside Felidae are collapsed, or (B) three genes combined, sampling only one individual per species. The sequences included in this analysis are found in Table S2. Support values on branches indicate Bayesian posterior clade probabilities/maximum likelihood bootstrap proportions. Stars represent nodes that were supported by BPP≥0.98 and MLBP≥95%; unnumbered clades received no support (BPP≤0.8, MLBP≤60).

Discussion

Palaeoecology

TSC is located ~60 km east of the Morland phase ice margin (Meyer & Knaeble 1998). This margin is correlated with the dated Verdi ice margin in southwestern Minnesota, *c.* 20 000 ^{14}C a BP (Clayton & Moran 1982), and the Sheldon Creek Formation in north-central Iowa, *c.* 40–26 000 ^{14}C a BP (Kilgore *et al.* 2007). Patterned ground indicative of a mean annual temperature below freezing is present throughout much of the Driftless Area for much of the late Wisconsin (Clayton *et al.* 2001; Iannicelli 2010). The close proximity of this mid-Wisconsin ice margin suggests that the area surrounding the cave would have been in permafrost and sparsely vegetated.

The vegetation, beetle faunas and molluscan faunas for this time period have no modern analogue, but indicate a cold, (mostly) treeless steppe-tundra environment. Further to the south, records from central Iowa and western Illinois indicate that the abrupt shift to open-land vegetation and beetle faunas occurred *c.* 22 700 ^{14}C a BP (Baker *et al.* 1989; Schwert 1992). The TSC *H. serum* habitat probably included open steppe-tundra uplands with a sparse spruce component in the lowlands and valleys.

The carnivore guild present in the Driftless Area during this time is virtually unknown. The herbivore component of the regional ecosystem, however, is dominated by megafauna. Stauffer's (1945) early work in Minnesota documented a significant number of *Mammuthus* localities for the southeastern corner of the state. Although poorly dated, the presence of early North American forms of *Mammuthus* (cf. *Mammuthus* (*Archidiskodon*) *imperator*) in Mississippi River gravel deposits suggests an extended time depth for this taxon. Large herbivores such as *Mammut americanum, Booth-*

erium bombifrons, *Bison* sp., *Rangifer* sp., *Platygonus compressus* and *Cervalces scotti* are also present in the Driftless Area (Hay 1923; Stauffer 1945; Palmer 1954; Long 1986; Theler *et al.* 1994; Hawley *et al.* 2007; Long & Yahnke 2011), but are poorly dated, or post-date the LGM.

The North American late Quaternary record of *H. serum* is sparse and highly dispersed. At Friesenhahn Cave (TX), *H. serum* was a specialized predator of juvenile mammoths (Meade 1961; Rawn-Schatzinger 1992). However, this assemblage also contains other herbivores (mastodons, peccaries, cervids, camels, bison, tapirs and horses), as well as a diverse range of carnivores (short-faced bear, black bear, dirk-toothed cat, jaguar, bobcat, dire wolf and coyote) (Graham 1976), albeit in much lower concentrations than *H. serum* and *Mammuthus*. Isotopic data from Beringia (Fox-Dobbs *et al.* 2008) and Rancho la Brea (Coltrain *et al.* 2004) suggest that *H. serum* diets were relatively generalized with regard to available large fauna. This is supported by reconstructions of *H. serum* bite behaviour (Wheeler 2011). Isotopic work hints that prey-choice in large felids may be strongly affected by other carnivores, especially large bears (Fox-Dobbs *et al.* 2008; Bocherens *et al.* 2011). Owing to the lack of recent work on Quaternary faunas of the Driftless Area, it is difficult to extend these generalizations to the palaeoecology of *H. serum* (and other members of the late Quaternary carnivore guild) in the upper Midwest.

Taxonomic and biogeographic implications for Homotherium serum

The combined genetic data (*cytb*+*ATP8*+12*S* r*RNA*) indicate that *Homotherium* and *Smilodon* are fairly divergent from one another (Fig. 9), consistent with their assignment to tribes Homotherini and Smilodontini, respectively. Genetic analyses support the monophyly of subfamily Machairodontinae, as it branches off before the divergence of extant felids (Martin 1998). Interestingly, the *cytb* data from two *Homotherium* specimens suggest that the individuals from TSC and Sixty Mile Creek, YT, Canada (CMN 46442; >56 500 ^{14}C a BP; Barnett *et al.* 2005) are very close genetically (identical at 408 of 410 bp) and belong to the same species. This extends the known range of *Homotherium serum* to the northeast within the United States and indicates reasonably low genetic variation between individuals sampled across a long geographic distance and separated by at least 30 000 years. This is in contrast to the case for another Pleistocene felid, the lion *Panthera leo*, in that the American lion, *P. leo atrox*, from the continental USA was genetically distinct from the Beringian cave lion, *P. leo spelaea*. However, like Homotherium, ancient *P. leo* individuals indicate low levels of intraspecific genetic diversity (Barnett *et al.* 2009).

Conclusions

This study describes the first occurrence of *Cervalces* sp. from Minnesota, and the first *H. serum* specimen from the upper Midwest and Great Lakes region. The age and location of the H. serum specimen suggest that this individual occupied a steppe-tundra environment. The morphology of the post-cranial skeleton is consistent with a moderate cursor that preyed upon the large herbivore fauna from the Driftless Area, *c.* 26 ka. Ancient DNA recovered from this individual confirms the identification of *H. serum* and illustrates that this species was widely spread across North America during the Pleistocene. Phylogenetic analyses show an early divergence between Smilodontini and Homotherini within Machairodontinae and support this subfamily as a distinct lineage within the Felidae prior to the divergence of extant Felinae and Pantherinae.

Acknowledgements. – The authors would like to acknowledge John Ackerman and Clay Kraus (Minnesota Karst Preserve), and David Mather (Minnesota Historical Society) for bringing this locality to our attention and supporting this research. Calvin Alexander (University of Minnesota), George Corner (University of Nebraska State Museum), Jeff Saunders, Terry Martin, Eric Grimm and Stacey Lengyel (Illinois State Museum) assisted with this project in various capacities. The Illinois State Museum Society provided funds for fieldwork and analysis. Matt Covington (Karst Research Institute) provided the map of Tyson Spring Cave. We thank reviewers M. Hofreiter, R. Barnett, and the editor J.A. Piotrowski for their helpful comments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Amplification primers and conditions.

Table S2. GenBank accession numbers and references for sequences included in this study.

Table S3. Tyson Spring Cave, *Cervalces* sp., cranial measurements.

Table S4. Summary of cranial measurements on large-cat taxa. Tyson Spring Cave (TSC); Friesenhahn Cave *H. serum* (FCV; from Rawn-Schatzinger 1992); Rancho la Brea *Smilodon* (RLB; Merriam & Stock 1932 and this study); Rancho la Brea *Panthera atrox* (Merriam & Stock 1932).

Table S5. Summary of scapula measurements on large-cat taxa. Tyson Spring Cave (TSC); Friesenhahn Cave *H. serum* (FCV; from Rawn-Schatzinger 1992); Rancho la Brea *Smilodon* (RLB; Merriam & Stock 1932 and this study); Rancho la Brea *Panthera atrox* (Merriam & Stock 1932); Texas *Panthera atrox* (FMNH P27071).

Table S6. Summary of humeral measurements on large-cat taxa. Tyson Spring Cave (TSC); Friesenhahn Cave *H. serum* (FCV; from Rawn-Schatzinger 1992); Rancho la Brea *Smilodon* (RLB; Merriam & Stock 1932 and this study); Rancho la Brea *Panthera atrox* (Merriam & Stock 1932); Texas *Panthera atrox* (FMNH P27071).